Effect of water stress on antioxidant systems and oxidative parameters in fruits of tomato (*Solanum lycopersicon* L, cv. Micro-tom)

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Abstract The effects of different levels of water stress on oxidative parameters (H_2O_2 and MDA), the total pool sizes of ascorbate, the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), as well as the activities and relative transcript levels of the enzymes of ascorbate-glutathione cycle ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) were studied in the fruit of tomato (*Solanum lycopersicum* L. cv. Micro-Tom). Plants were subjected to three levels of water stress (S50, S25 and S0) and fruits at different development stages were harvested after 3, 6 and 10 days of stress. Changes in H_2O_2 and MDA contents indicated that water stress induced oxidative stress in fruits. The concentrations of ascorbate (AsA) and dehydroascorbate (DHA) generally modified with water stress treatments. Moreover, changes in SOD and CAT activities and DHAR, MDHAR, APX and GR activities and relative transcript levels were dependent on the fruit development stage and the intensity and the duration of water stress. These results suggest that the response of antioxidant systems of tomato fruits to oxidative stress induced by water stress treatments was different depending on the fruit development stage.

Keywords Antioxidant enzymes · Ascorbate · Water stress · Oxidative stress · *Solanum lycopersicum* L.

Introduction

Drought is one of the most important environmental factors that influence plant growth and development, and limit plant production. Decreased water availability has an immediate impact on water status and affects plant growth via detrimental effects on water absorption, photosynthesis and the transport of water and solutes to growing organs like fruit. Plants can respond and adapt to water stress by altering their cellular metabolism and invoking various defence mechanisms (Bohnert and Jensen 1996). Drought leads to oxidative stress in the plant cell due to higher leakage of electrons towards O_2 during photosynthetic and respiratory processes leading to enhancement in reactive oxygen species (ROS) generation (Asada 1999; Sánchez-Rodríguez et al. 2012). ROS are by-products of aerobic metabolism and their production is enhanced during water stress conditions through the disruption of electron transport system and oxidizing metabolic activities occurring in chloroplasts, mitochondria and microbodies (Asada 1999; Van Breusegem et al. 2001; Ahmad et al. 2010). ROS, such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$) and singlet oxygen (1O_2), are damageable for cellular structures and macromolecules, causing photoinhibition of photosynthetic apparatus (Smirnoff 1993; Ahmad et al. 2011). Also, its can directly attack membrane lipids, inactivate metabolic enzymes and damage the nucleic acids leading to cell death (Mittler 2002; Gill and Tuteja 2010; Ahmad et al. 2011).

During optimal conditions, the balance between ROS formation and consumption is tightly controlled by non-enzymatic and enzymatic antioxidants. Whereas, during drought conditions, the production of ROS exceeds the capacity of the antioxidative systems to remove them, causing oxidative stress (Noctor and Foyer 1998). The non-enzymatic antioxidant system includes ascorbate (AsA),

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glutathione (GSH), carotenoids (lycopene, carotene), α -tocopherol and many other phenolic compounds. AsA is a major primary antioxidant synthesized on the inner membrane of the mitochondria which reacts chemically with $^1\text{O}_2$, $\text{O}_2^{\cdot-}$, $\text{HO}^{\cdot}$ and thiol radical (Noctor and Foyer 1998), and acts as the natural substrate of many plant peroxidases (Mehlhorn et al. 1996). Moreover, AsA is involved in other functions such as plant growth, gene regulation, and modulation of some enzymes and redox regulation of membrane-bound antioxidant compounds (Horemans et al. 2000). The antioxidant enzymatic system includes superoxide dismutase (SOD), catalase (CAT) and the enzymes of the ascorbate–glutathione cycle that operates both in the chloroplast and in the cytosol: ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). The first enzyme involved in the ROS scavenging system is SOD, that catalyzes the dismutation of the superoxide anion ($\text{O}_2^{\cdot-}$) to oxygen and hydrogen peroxide (H_2O_2) (Gomez et al. 2004). H_2O_2 was then converted into non-toxic water by CAT or APX (Asada 2006). The enzyme APX is implicated in oxidation of AsA to monodehydroascorbate (MDHA), which can either dismutate back to AsA, or to dehydroascorbate (DHA). The DHA is then rapidly reduced to AsA (Asada 2006). GR enzyme is involved in the regeneration of AsA from MDHA via the NADPH-dependent reduction of oxidized GSSG to the reduced GSH (Noctor et al. 2002; Gill and Tuteja 2010).

Water stress, like other abiotic stress, is generally associated with increased oxidative stress due to the enhanced production of ROS, particularly $\text{O}_2^{\cdot-}$ and H_2O_2 (Scandalios 1997). The active ascorbate–glutathione cycle is responsible for efficient removal of excess ROS. AsA can directly scavenge the H_2O_2 and thus protect the integrity of various membranes by preventing the conversion of H_2O_2 into highly potent $\text{OH}^{\cdot}$ radicals, which could induce lipid peroxidation leading to membrane damage (Navari-Izzo et al. 1994). APX, in presence of AsA, is involved in H_2O_2 scavenging and minimizes the subsequent oxidative conditions in the cell. The level of ascorbate–glutathione and the activities of antioxidant enzymes are generally increased during abiotic stress conditions and correlate with enhanced cellular protection (Asada 2006; Duan et al. 2012).

Tomato plants are highly susceptible to water deficit notably during the growth phase, but also at flowering and the fruit growth. Withholding water decreases leaf and fruit size, fruit number, the rate of photosynthesis and fruit quality (Dodds et al. 1997). In tomato plants, the changes in the antioxidant systems in response to different stresses have been investigated in detail in leaves, and there is a considerable amount of literature concerning leaf responses to water stress with respect to oxidative stress defence

mechanisms. However, little is known about such response mechanisms in fleshy fruits subjected to drought. Pericarp of tomato fruits initially contains chloroplasts that are photosynthetically active, but they differentiate to non-photosynthetic chromoplasts during the ripening process. So, depending on the age of fruits, chloroplast photosynthesis or mitochondria respiration is a primary source of superoxide (Purvis et al. 1995).

The aim of the present study is to investigate the effect of water deficit on some oxidative parameters and the antioxidant systems in the fruits of tomato variety Micro-Tom at different development stages. We thus measured H_2O_2 content, membrane injury, the total pool sizes of ascorbate as well as the activities and relative transcript levels of the enzymes of ascorbate–glutathione cycle.

Materials and methods

Plant material and treatments

Tomato (*Solanum lycopersicum* L, cv. Micro-tom) seeds were germinated in boxes filled with peat. Then seedlings were transplanted to 4 L plastic containers containing a mixture of peat and vermiculite (1:1). Plants were grown in a growth room at a relative humidity of $60 \pm 5\%$ and with day/night temperature of $25/20^\circ\text{C}$ using a 16/8 h light/dark cycle under $300\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ fluorescent light. At the time of anthesis, flowers were tagged for determining fruit age. Plants were grouped into four sets and subjected to water stress treatments: First set (C), control plants were irrigated daily with an amount of water equal to the evapo-transpired water. Second set (S50) and third set (S25), plants were irrigated daily with an amount of water equal to 50 and 25 % of the evapo-transpired water, respectively. And, fourth set (S0) plants were not irrigated during stress period. The fruits were harvested after 3, 6 and 10 days of treatment, between 9 and 10 AM and they were grouped depending on development stage into: small green (25, 30, 35 days after flowering; DAF), mature green (fully developed green fruit, 40 DAF), breaker (2–3 days after mature green stage), orange (2–3 days after breaker stage) and red (4–6 days after orange stage). For each stage and treatment, fruits were harvested from three plants and immediately frozen in liquid nitrogen, ground and the powder was stored at -80°C .

Determination of H_2O_2 content

Hydrogen peroxide levels were determined as described by Murshed et al. (2008a). Frozen fruit powder (0.25 g) was homogenized in an ice bath with 1 mL 0.1 % (w:v) TCA. The homogenate was centrifuged at $12\ 000g$ for 15 min at 4°C . Aliquots of 100 μL from each tube were placed in 96-

well plates and 50 μL of 10 mM potassium phosphate buffer (pH 7.0) and 100 μL of 1 M KI were added to each well. Commercial H_2O_2 was used to generate a standard curve. The plate was briefly vortexed, incubated at room temperature for 30 min. and the absorbance readings were taken at 390 nm in a micro-plate reader. The content of H_2O_2 was determined using the standard curve.

Determination of the malonyldialdehyde content

For the measurement of lipid peroxidation in fruits, the thiobarbituric acid (TBA) test, which determines malonyldialdehyde (MDA) as an end product of lipid peroxidation (Murshed et al. 2008a), was used. Frozen fruit powder (0.25 g) was homogenized in 1 mL 0.1 % (w:v) TCA solution. The homogenate was centrifuged at 12 000 g for 15 min and 0.5 mL of the supernatant was added to 1 mL 0.5 % (w:v) TBA in 20 % TCA. The mixture was incubated in boiling water for 30 min, and the reaction stopped by placing the reaction tubes in an ice bath. Tubes were briefly vortexed, triplicate, 200 μL aliquots from each tube were placed in 96-well plates, and the absorbance of supernatant was read at 532 nm in a micro-plate reader. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA–TBA complex (red pigment) was calculated using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Determination of ascorbate and dehydroascorbate contents

Total ascorbate (AsA plus DHA) and AsA contents were measured according to the method of Kampfenkel et al. (1995), scaled down for micro-plates (Murshed et al. 2008a). A 0.5 g sample of frozen ground tomato fruit was homogenized in 1 mL of cold 6 % (w:v) trichloroacetic acid (TCA). The homogenate was centrifuged at 16 000 g at 4 °C for 15 min. The supernatant was used for total ascorbate and AsA determination.

For measurements of total ascorbate, 10 μL of extract were added to 10 μL of 10 mM DL-dithiothreitol (DTT) and 20 μL of 0.2 mM phosphate buffer (pH 7.4). After incubation for 15 min at 42 °C, 10 μL of 0.5 % (w:v) *N*-ethylmaleimide (NEM) was added with additional incubation for 1 min at room temperature to remove excess DTT. This was followed by adding 150 μL of a reagent prepared just before use by mixing 50 μL of 10 % TCA, 40 μL of 42 % (v:v) of orthophosphoric acid (H_3PO_4), 40 μL of 4 % (w:v) 2,2-bipyridyl dissolved in ethanol (70 %) and 20 μL of 3 % (w:v) ferric chloride. After further incubation for 40 min at 42 °C, the absorbance was measured at 525 nm in a micro-plate reader. For AsA determination, the same reaction was used but 0.2 M phosphate buffer (pH 7.4) was used in place of DTT and NEM. The amount of DHA was estimated from the difference of total ascorbate and AsA.

Commercial L-Ascorbic acid was used to generate a standard curve.

Antioxidant enzyme assays

Extraction of enzymes

Protein extraction was performed according to the Murshed et al. (2008b) method. Frozen fruit powder (0.20 to 0.4 g) was homogenized in 1 ml of 50 mM MES/KOH buffer (pH 6.0), containing 40 mM KCl, 2 mM CaCl_2 , and 1 mM AsA. Extracts were centrifuged at 4 °C for 15 min at 16 000 g, and the supernatants were analysed immediately for enzyme activities. Protein was quantified using Bradford's method (Bradford 1976).

Enzyme assays

All enzyme activities were measured in 200 μL volume kinetic reactions at 25 °C, using a micro-plate reader. APX, DHAR, MDHAR and GR activities were measured as described in the method of Murshed et al. (2008b). SOD activity was assayed in a 1.0 ml reaction mixture containing 50 mM potassium phosphate (pH 7.8) buffer, 13 mM methionine, 75 μM nitro blue tetrazolium (NBT), 0.1 mM EDTA, 10 μL of sample supernatant and 2 μM riboflavin. Tubes were briefly vortexed and triplicate, 200 μL aliquots from each tube, were placed in plastic 96-well plates. Commercial SOD was used to generate a standard curve. The plates were then placed under white light provided by a commercial overhead-transparency projector (Horizon, Model Apollo, Lincolnshire, IL, USA) for 5 min. After the light treatment, absorbance readings were taken at 560 nm in a plate reader. This method was modified from that of Dhindsa et al. (1981). CAT activity was measured using a method adapted from that of Aebi (1984) in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 20 μL of sample supernatant. Activity was determined by measuring the decrease in the reaction rate at 240 nm and calculated using a $43.6 \text{ M}^{-1} \text{ cm}^{-1}$ extinction coefficient.

RNA extraction, cDNA synthesis and quantitative RT-PCR detection

Total RNA was isolated from 50 mg to 100 mg of frozen fruit powder using Tri Reagent (MRC, Molecular Research Center, INC) following to manufacturer's instructions. RNAs were quantified spectrophotometrically measuring the absorbance at 260 and 280 nm. RNA integrity was verified on 1 % agarose gels.

For RT-PCR experiments, first-strand cDNA was synthesized from 4 μg of total RNA using the Masterscript™ RT-

PCR system (5 PRIME) following to manufacturer's instructions. After the initial template RNA denaturation at 65 °C for 5 min, the first-strand cDNA was synthesized using Oligo d(T) primer at 42 °C for 60 min, and then the reaction was interrupted by enzyme inactivation at 85 °C for 5 min.

The quantitative assessment of mRNA levels was performed using the RealMasterMix SYBR ROX (5 PRIME) in MastercyclerRealplex (Eppendorf). The quantification of the accumulation of the target transcript relative to the actin (*SlActin*) transcript, taken as the control gene for the experimental conditions, was performed with specific primers for each gene. Specific primers pairs of *SlActin*, *SLAPXcyto*, *SLAPXt*, *SIDHAR1*, *SIDHAR2*, *SIMDHAR*, *SIGRcyto* and *SIGRcp* were designed based on sequences present in the GenBank database (<http://www.ncbi.nlm.nih.gov>) using DNAMAN software (Table 1). Three independent RNA isolations, with two replications for each of the RNA isolations, were performed for each stage and treatment. Amplified fragments of PCR product were purified using the QIAquick PCR Purification Kit (QIAGEN) following to manufacturer's instructions, and sequenced using Genome Express services. Sequences data have been deposited with the GenBank database under accession numbers referenced in Table 1.

Statistic analysis

The experiment has been repeated three times, and the values presented here are the means of the three experiments. The results are expressed as means with standard error ($\pm$ SE). Statistical significance was determined by analysis of variance ($p < 0.05$) using MINITAB Release 14 Statistical Software.

Results

H₂O₂ content and lipid peroxidation

The malondialdehyde (MDA) assay has been used to estimate peroxidation of lipids in membrane to determine if oxidative damages appeared during fruit development. H₂O₂ content has been quantified as indicator of ROS accumulation. H₂O₂ content and membrane lipid peroxidation are shown in Tables 2 and 3, respectively. Most water treatments increased the H₂O₂ content during fruit growth up to the mature green stage. However, H₂O₂ content decreased in fruits at other ripening stages of S25 and S0 stressed for 6 and 10 days (Table 2). The MDA content in fruits during fruit growth and ripening increased significantly under some water treatments, except in fruits at 35 DAF and mature green stage where it not changed under water stress (Table 3).

Non-enzymatic antioxidants

The effects of drought on AsA and DHA concentrations in fruits are shown in Figs. 1 and 2, respectively. Most water treatments increased the AsA concentration during fruit development, except a decrease in AsA concentration in S25 fruits at 35 DAF stressed for 3 days (Fig. 1a) and in S0 fruits at breaker stage stressed for 6 days (Fig. 1b). DHA concentration also increased under some water treatments, but it decreased under some other water treatments (Fig. 2).

Antioxidant enzyme activities

Effect of water stress on activities of SOD and CAT during fruit growth and ripening were established in Fig. 3. SOD

Table 1 Sets of primers used to amplify gene-specific regions, corresponding size and accession number of the amplified product

PCR fragment	Encodedprotein	Primer sequence (5'→3')	Size (bp)	Accession number
<i>SLAPXt</i>	Thylakoid-bound ascorbate peroxidase	Sense : TTCACCCAATGACTTCCCT Antisense:TATCATTAGTCCCATTCTGT	699	FJ532352
<i>SLAPXcyto</i>	Cytosolic ascorbate peroxidase	Sense : GTTGAAGGTCGCTTGCCG Antisense:CCAAGGTATGGGCACCAG	118	FJ532353
<i>SIDHAR1</i>	Dehydroascorbate reductase	Sense : TGCCTCTGTGGGCTCGAA Antisense:ACCACCCTGCGATGACGT	335	FJ532354
<i>SIDHAR2</i>	Dehydroascorbate reductase	Sense : ACAACTCCTAACAAGCTCGG Antisense:GTCCAAGCGACAAATCAGCA	430	FJ532355
<i>SIMDHAR</i>	Monodehydroascorbate reductase	Sense : GGAGAAGTTTCGTTGCTGCT Antisense:TGAGCAGCTTTCCTGAATTGT	371	FJ544908
<i>SIGRcp</i>	Chloroplastic glutathione reductase	Sense : TGGAGGCGTCGGTGGCAC Antisense:CTATAGCATACTCGTTCCAG	362	FJ544906
<i>SIGRcyto</i>	Cytosolic glutathione reductase	Sense : GCAAAGAATTATGGATGGGA Antisense:CACAGCACGCTTTGGTAA	333	FJ544907
<i>SlActin</i>	Actin	Sense :ATGACTCAAATCATGTTTGTGAG Antisense:TACCTTAATCTTCATGCTGCT	633	FJ532351

Table 2 H₂O₂ (nmol g⁻¹ FW) content in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3, 6 and 10 days

	3D					6D					10D				
	C	S50	S25	S0	C	S50	S25	S0	C	S50	S25	S0	C	S50	S0
25 DAF	274.63±48.2 ^a	306.29±38.2 ^a	494.47±30.9 ^b	690.74±52.7 ^c	274.63±48.2 ^a	272.03±30.6 ^a	354.28±26.8 ^a	509.65±46.8 ^b	274.63±48.2 ^a	286.36±22.0 ^a	472.75±50.7 ^b	408.43±31.5 ^b	274.63±48.2 ^a	286.36±22.0 ^a	472.75±50.7 ^b
30 DAF	108.49±6.9 ^a	181.11±28.8 ^b	458.98±58.4 ^c	427.45±82.1 ^c	108.49±6.9 ^a	330.91±63.9 ^b	470.36±36.4 ^c	498.05±16.6 ^c	108.49±6.9 ^a	472.94±59.7 ^b	502.16±76.2 ^b	535.14±64.0 ^b	108.49±6.9 ^a	472.94±59.7 ^b	502.16±76.2 ^b
35 DAF	82.66±6.8 ^a	89.92±14.3 ^a	302.23±20.8 ^b	348.95±17.3 ^b	82.66±6.8 ^a	104.06±4.3 ^b	440.06±17.3 ^c	512.76±23.3 ^d	82.66±6.8 ^a	129.56±15.2 ^b	633.85±26.7 ^c	537.54±23.2 ^d	82.66±6.8 ^a	129.56±15.2 ^b	633.85±26.7 ^c
Mature green	35.68±2.2 ^a	37.76±1.4 ^a	77.91±1.7 ^b	89.35±1.8 ^c	35.68±2.2 ^a	42.47±1.1 ^b	103.40±4.7 ^c	120.33±3.2 ^d	35.68±2.2 ^a	44.82±2.1 ^b	131.06±2.7 ^c	133.69±2.4 ^c	35.68±2.2 ^a	44.82±2.1 ^b	131.06±2.7 ^c
Breaker	4.68±0.9 ^a	6.43±0.9 ^a	4.43±0.5 ^a	4.08±0.3 ^a	4.68±0.9 ^a	3.73±0.8 ^a	2.70±0.3 ^b	2.29±0.2 ^b	4.68±0.9 ^a	1.27±0.1 ^b	1.90±0.2 ^b	1.18±0.2 ^b	4.68±0.9 ^a	1.27±0.1 ^b	1.90±0.2 ^b
Orange	23.04±1.8 ^a	24.49±1.7 ^a	22.00±1.1 ^a	23.06±1.2 ^a	23.04±1.8 ^a	20.97±1.3 ^a	17.89±0.9 ^b	8.85±0.7 ^c	23.04±1.8 ^a	21.17±0.4 ^a	4.59±0.3 ^b	5.75±0.6 ^b	23.04±1.8 ^a	21.17±0.4 ^a	4.59±0.3 ^b
Red	54.07±2.5 ^a	55.41±2.8 ^a	54.30±1.89 ^a	53.46±3.4 ^a	54.07±2.5 ^a	52.93±3.4 ^a	46.25±1.1 ^b	32.80±1.0 ^c	54.07±2.5 ^a	54.28±2.7 ^a	34.61±0.7 ^b	24.49±1.3 ^c	54.07±2.5 ^a	54.28±2.7 ^a	34.61±0.7 ^b

Values are the mean (±S.E.) of five replicates, and different letters within lines indicate significant differences ($P<0.05$). DAF days after flowering

Table 3 Malonyldialdehyde (MDA; nmol g⁻¹ FW) content in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3, 6 and 10 days

	3D					6D					10D				
	C	S50	S25	S0	C	S50	S25	S0	C	S50	S25	S0	C	S50	S0
25 DAF	24.24±1.5 ^a	24.59±2.6 ^a	25.02±1.5 ^a	35.48±1.9 ^b	24.24±1.5 ^a	28.57±0.9 ^b	23.81±0.4 ^a	28.01±1.8 ^b	24.24±1.5 ^a	25.43±1.6 ^a	26.34±1.9 ^a	23.16±0.8 ^a	24.24±1.5 ^a	25.43±1.6 ^a	26.34±1.9 ^a
30 DAF	24.86±1.8 ^a	25.81±1.4 ^a	23.08±1.9 ^a	22.23±1.6 ^a	24.86±1.8 ^a	29.78±1.4 ^b	29.29±1.9 ^b	24.93±1.0 ^a	24.86±1.8 ^a	27.39±1.9 ^a	23.23±1.5 ^a	32.96±1.3 ^b	24.86±1.8 ^a	27.39±1.9 ^a	23.23±1.5 ^a
35 DAF	24.08±0.3 ^a	24.20±1.1 ^a	23.97±0.5 ^a	22.66±1.3 ^a	24.08±1.3 ^a	24.24±0.8 ^a	22.33±1.5 ^a	25.31±2.1 ^a	24.08±0.3 ^a	23.08±2.6 ^a	24.83±2.3 ^a	25.12±1.9 ^a	24.08±0.3 ^a	23.08±2.6 ^a	24.83±2.3 ^a
Mature green	23.63±1.2 ^a	24.22±1.4 ^a	22.85±1.4 ^a	24.04±1.0 ^a	23.63±1.2 ^a	25.70±1.5 ^a	22.37±1.7 ^a	25.79±0.9 ^a	23.63±1.2 ^a	25.91±1.5 ^a	22.97±0.7 ^a	24.17±2.5 ^a	23.63±1.2 ^a	25.91±1.5 ^a	22.97±0.7 ^a
Breaker	20.20±1.8 ^a	22.99±1.4 ^a	19.90±0.9 ^a	24.44±1.2 ^b	20.20±1.8 ^a	26.79±1.5 ^b	20.44±0.9 ^a	24.65±0.7 ^b	20.20±1.8 ^a	20.18±1.7 ^a	19.60±1.3 ^a	18.16±1.2 ^a	20.20±1.8 ^a	20.18±1.7 ^a	19.60±1.3 ^a
Orange	22.67±1.2 ^a	22.85±1.1 ^a	24.76±1.0 ^a	26.49±1.6 ^b	22.67±1.2 ^a	26.07±0.9 ^b	23.97±1.4 ^a	32.65±1.8 ^c	22.67±1.2 ^a	22.44±1.6 ^a	22.42±0.9 ^a	28.28±1.3 ^b	22.67±1.2 ^a	22.44±1.6 ^a	22.42±0.9 ^a
Red	40.69±1.8 ^a	46.13±1.6 ^b	46.94±1.9 ^b	44.99±1.1 ^b	40.69±1.8 ^a	42.75±1.9 ^a	42.57±1.9 ^a	40.42±1.6 ^a	40.69±1.8 ^a	41.66±2.3 ^a	52.76±3.1 ^b	46.79±1.4 ^c	40.69±1.8 ^a	41.66±2.3 ^a	52.76±3.1 ^b

Values are the mean (±S.E.) of five replicates, and different letters within lines indicate significant differences ($P<0.05$). DAF days after flowering

Fig. 1 Ascorbate (AsA) concentration (nmol. g⁻¹ FW) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3, 6 and 10 days (**a**, **b** and **c**, respectively). Values are the mean ($\pm$ S.E.) of five replicates. DAF days after flowering

activity was increased in fruits at 35 DAF, mature green and breaker stages by water stress treatments. However, in fruits at 25 and 30 DAF, orange and red stages SOD activity was not modified by water stress treatments (Fig. 3a). CAT activity in fruits was increased by water stress treatments, except in all fruits at 35 DAF stressed for 10 days, in S0 fruits at the red stage stressed for 3 days, in S50 and S0 fruits stressed for 6 days and in S25 and S0 fruits stressed for 10 days, where it was decreased, and in fruits at the orange stage where CAT activity was not changed by water stress treatments (Fig. 3b).

Fig. 2 Dehydroascorbate (DHA) concentration (nmol. g⁻¹ FW) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3, 6 and 10 days (**a**, **b** and **c**, respectively). Values are the mean ($\pm$ S.E.) of five replicates. DAF days after flowering

Effects of water stress on activities of enzymes associated with the ascorbate-glutathione cycle (APX, MDHAR, DHAR and GR) were presented in Fig. 4. APX activity was increased by most water stress treatments; however, it was decreased in S0 fruits at the orange stage stressed for 10 days (Fig. 4a). DHAR activity was also increased by some water stress treatments; but it was decreased in all fruits at 35 DAF stressed for 10 days (Fig. 4b). MDHAR activity was increased by some water stress treatments, in contrast, a decrease in MDHAR activity was found in all fruits at the breaker stage stressed for 3 days, in S0 fruits at

Fig. 3 **a** Activity of superoxide dismutase (SOD; **a**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1* and *b1*), 6 days (*a2* and *b2*) and 10 days (*a3* and *b3*). Values are the mean ($\pm$ S.E.) of five replicates. DAF days after flowering. **b** Activity of

catalase (CAT; **b**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1* and *b1*), 6 days (*a2* and *b2*) and 10 days (*a3* and *b3*). Values are the mean ($\pm$ S.E.) of five replicates. DAF days after flowering

the breaker stage stressed for 6 days and in S50 fruits at orange stage stressed for 3 days (Fig. 4c). GR activity was increased by most water stress treatments (Fig. 4d).

Gene expression of antioxidant enzymes

In addition to enzyme activity study, the relative transcript levels of some genes encoding these enzymes were determined in fruit during development and under different water deficit treatments using real-time quantitative RT-PCR. Among the ascorbate-glutathione cycle genes, the following transcript were chosen: *SLAPXcyto*, *SLAPXt*, *SIMDHAR*, *SIDHAR1*, *SIDHAR2*, *SIGRcyto* and *SIGRcp* genes encoding for tomato cytosolic and thylakoid-bound APX, MDHAR, DHAR, cytosolic and chloroplastic GR, respectively. The

relative transcript levels were measured in fruits of control and treated plants and normalized to transcript levels of *SLActin*. Data were expressed as relative values with respect to the value found in the control fruits and presented in Fig. 5.

The *SLAPXcyto* transcript level increased by most water stress treatments in fruits at all development stages (Fig. 5a). *SLAPXt* transcript level increased in all fruits of plants stressed for 3 days except in S50 fruits at 25, 30 and 35 DAF, in S25 fruits at 25 DAF and in S0 fruits at red stage, where no change in *SLAPXt* transcript level was observed and in S0 fruits at 25 DAF and in S25 and S0 fruits at 30 DAF, where a decrease in levels of *SLAPXt* transcript was found (Fig. 5b1). In plants stressed for 6 days, *SLAPXt* transcript increased in all fruits except in S50 fruits at 25,

Fig. 4 **a** Activity of ascorbate peroxidase (APX; **a**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1*, *b1*, *c1* and *d1*), 6 days (*a2*, *b2*, *c2* and *d2*) and 10 days (*a3*, *b3*, *c3* and *d3*). Values are the mean ($\pm$ S.E.) of five replicates. DAF days after flowering. **b** Activity of dehydroascorbate reductase (DHAR; **b**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1*, *b1*, *c1* and *d1*), 6 days (*a2*, *b2*, *c2* and *d2*) and 10 days (*a3*, *b3*, *c3* and *d3*). Values are the mean ($\pm$ S.E.) of five replicates. DAF days after flowering. **c** Activity of monodehydroascorbate

(MDHAR; **c**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1*, *b1*, *c1* and *d1*), 6 days (*a2*, *b2*, *c2* and *d2*) and 10 days (*a3*, *b3*, *c3* and *d3*). Values are the mean ($\pm$ S.E.) of five replicates. DAF days after flowering. **d** Activity of glutathione reductase (GR; **d**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1*, *b1*, *c1* and *d1*), 6 days (*a2*, *b2*, *c2* and *d2*) and 10 days (*a3*, *b3*, *c3* and *d3*). Values are the mean ($\pm$ S.E.) of five replicates. DAF days after flowering

30 and 35 DAF, where no change in *SLAPXt* transcript level was observed and in S0 fruits at 25 DAF, in S25 and S0 fruits at 30 DAF and in all fruits at red stage, where it decreased (Fig. 5b2). In plants stressed for 10 days, *SLAPXt* transcript increased in all fruits except S50 fruits at 25 DAF, where no change in *SLAPXt* transcript level was observed and in S25 and S0 at 25 DAF, in all fruits at 30 DAF and in S50 fruits at the red stage, where it decreased (Fig. 5b3).

SIDHAR1 transcript increased in all fruits of plants stressed for 3 days except in S0 fruits at 25 DAF, breaker and red stages, where it not changed and in S0 fruits at 35 DAF, where

a decrease in *SIDHAR1* transcript was found (Fig. 5c1). In plants stressed for 6 days, *SIDHAR1* transcript increased in all fruits except in fruits at 35 DAF, where it shows no change in S50 fruits but decreased in S25 and S0 fruits (Fig. 5c2). In plants stressed for 10 days, *SIDHAR1* transcript increased in all fruits except in S50 and S25 fruits at 25 DAF, where it shows no change and in S0 fruits at 35 DAF, where it decreased (Fig. 5c3). *SIDHAR2* transcript increased in all fruits of plants stressed for 3 days except in S0 fruits at 35 DAF, where it shows no change (Fig. 5d1). In plants stressed for 6 days, *SIDHAR2* transcript increased in all fruits except in S0

Fig. 4 (continued)

fruits at 35 DAF, where it decreased and in S25 and S0 at breaker stage, where it decreased (Fig. 5d2). In plants stressed for 10 days, *SIDHAR2* transcript increased in all fruits except in S25 fruits at 35 DAF and S0 fruits at breaker stage, where it shows no change and in S0 fruits at 35 DAF, where it decreased (Fig. 5d3). *SIMDHAR* transcript increased in all fruits of plants stressed except in all fruits at 25 DAF, where it decreased and in S50 fruits at mature green and red stages, where it not changed (Fig. 5e).

SIGRcyto transcript increased in all fruits of plants stressed for 3 days except in S50 and S0 fruits at 25 DAF and in S50 fruits at mature green, breaker and red stages, where it shows no change and in all fruits at 35 DAF and in S25 and S0 fruits at breaker stage, where it decreased (Fig. 5f1). In plants stressed for 6 days, an increase in *SIGRcyto* transcript was found in all fruits except in S0 fruits at 35 DAF, where a decrease was found and in S50

fruits at red stage, where it shows no change (Fig. 5f2). In plants stressed for 10 days, *SIGRcyto* transcript increased in all fruits except in S50 fruits at 25 DAF and red stage, where it not changed and in S0 fruits at 25 DAF and in all fruits at 35 DAF, where it decreased (Fig. 5f3).

SIGRcp transcript increased in all fruits of plants stressed for 3 days except in S0 and S25 fruits at 25 DAF, S25 fruits at breaker stage, S0 fruits at orange stage and S50 and S0 fruits at red stage, where it shows no change and in S0 fruits at 25 DAF, S50 and S25 fruits at 35 DAF and S0 fruits at orange, where it decreased (Fig. 5g1). In plants stressed for 6 days, an increase in *SIGRcp* transcript was found in all fruits except in S50 and S25 fruits at 25 DAF, in S0 fruits at 35 DAF, breaker and orange stages and in S50 and S0 fruits at red stage, where *SIGRcp* transcript not changed and in S0 fruits at 25 DAF, where it decreased (Fig. 5g2). In plants stressed for 10 days, *SIGRcp* transcript increased in all fruits

Fig. 5 **a** Changes in relative transcript level of cytosolic APX (*SlAPXcyto*; **a**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1, b1, c1, d1, e1, f1* and *g1*), 6 days (*a2, b2, c2, d2, e2, f2* and *g2*) and 10 days (*a3, b3, c3, d3, e3, f3* and *g3*). Data were expressed as relative values with respect to the value found in the control fruits. Values are the mean ($\pm$ S.E.) of nine replicates. DAF days after flowering. **b** Changes in relative transcript level of thylakoid-bound APX (*SlAPXt*; **b**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1, b1, c1, d1, e1, f1* and *g1*), 6 days (*a2, b2, c2, d2, e2, f2* and *g2*) and 10 days (*a3, b3, c3, d3, e3, f3* and *g3*). Data were expressed as relative values with respect to the value found in the control fruits. Values are the mean ($\pm$ S.E.) of nine replicates. DAF days after flowering. **c** Changes in relative transcript level of *SIDHAR1* (**c**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1, b1, c1, d1, e1, f1* and *g1*), 6 days (*a2, b2, c2, d2, e2, f2* and *g2*) and 10 days (*a3, b3, c3, d3, e3, f3* and *g3*). Data were expressed as relative values with respect to the value found in the control fruits. Values are the mean ($\pm$ S.E.) of nine replicates. DAF days after flowering. **d** Changes in relative transcript level of *SIDHAR2* (**d**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1, b1, c1, d1, e1, f1* and *g1*), 6 days (*a2, b2, c2, d2, e2, f2* and *g2*) and 10 days (*a3, b3, c3, d3,*

e3, f3 and *g3*). Data were expressed as relative values with respect to the value found in the control fruits. Values are the mean ($\pm$ S.E.) of nine replicates. DAF days after flowering. **e** Changes in relative transcript level of *SIMDHAR* (**e**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1, b1, c1, d1, e1, f1* and *g1*), 6 days (*a2, b2, c2, d2, e2, f2* and *g2*) and 10 days (*a3, b3, c3, d3, e3, f3* and *g3*). Data were expressed as relative values with respect to the value found in the control fruits. Values are the mean ($\pm$ S.E.) of nine replicates. DAF days after flowering. **f** Changes in relative transcript level of cytosolic GR (*SlGRcyto*; **f**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1, b1, c1, d1, e1, f1* and *g1*), 6 days (*a2, b2, c2, d2, e2, f2* and *g2*) and 10 days (*a3, b3, c3, d3, e3, f3* and *g3*). Data were expressed as relative values with respect to the value found in the control fruits. Values are the mean ($\pm$ S.E.) of nine replicates. DAF days after flowering. **g** Changes in relative transcript level of chloroplastic GR (*SlGRcp*; **g**) in fruits of control plants (C) and plants stressed (S50, S25 and S0) for 3 days (*a1, b1, c1, d1, e1, f1* and *g1*), 6 days (*a2, b2, c2, d2, e2, f2* and *g2*) and 10 days (*a3, b3, c3, d3, e3, f3* and *g3*). Data were expressed as relative values with respect to the value found in the control fruits. Values are the mean ($\pm$ S.E.) of nine replicates. DAF days after flowering

Fig. 5 (continued)

except in all fruits at 25 DAF and S50 fruits at red stage, where it shows no change and in S50 fruits at 35 DAF and in S0 fruits at orange stage, where *SIGRcp* transcript decreased (Fig. 5g3).

Discussion

Drought is the major limiting factor in many parts of the world, which seriously affects plant growth and yield (Shao et al. 2007). Plant experiences drought either when the water supply to roots becomes difficult or when the transpiration rate becomes very high (Sankar et al. 2007). Water stress adversely affects many physiological and biochemical process in plants (Reddy et al. 2004; Manivannan et al.

2007a, b). The reactions of the plants to water stress differ significantly at various organizational levels depending on intensity and duration of stress as well as plant species and its stage of development (Chaves et al. 2003). Understanding plant responses to water stress is of great importance and also fundamental parts for making the crops stress tolerant (Reddy et al. 2004). In the present study, tomato plants were subjected to three different levels of water stress to evaluate the effects of water stress on oxidative parameters and antioxidant defence mechanisms in fruits. In addition, water deficit treatments were applied at different stage of fruits development to analyse the impact of stress in function of days after anthesis. As age of fruits at harvesting has seen more interesting than age at day of treatment, all results are presented

Fig. 5 (continued)

in function of age of fruits at the harvest in days after flowering (DAF). The fruit oxidative parameters (H_2O_2 and MDA) were determined. The mechanisms that confer protection to oxidative stress were also investigated. For this purpose, AsA and DHA concentrations and the activities of antioxidant enzymes: SOD and CAT, as well as the activities and relative transcript levels of the enzymes of ascorbate-glutathione cycle (APX, DHAR, MDHAR and GR) were analysed.

The H_2O_2 and MDA contents were measured in fruits as an indicator of oxidative stress (Mittler 2002). H_2O_2 is involved in virtually all major areas of aerobic biochemistry (e.g. respiratory and photosynthetic electron transport; oxidation of glycolate, xanthine, and glucose) and is produced in copious quantities by several enzyme systems, even under optimal conditions (Noctor and Foyer 1998). Water stress induces oxidative stress because of inhibition of photosynthetic activity due to imbalance between light capture

and its utilization (Foyer and Noctor 2005). Our results showed an increase in the H_2O_2 content in fruits with water stress during fruit growth. Whereas, H_2O_2 content decreased in fruits at ripening stages (Table 2). These different responses of H_2O_2 content to water stress between fruit development stages can be explained, in part, by a lower formation of H_2O_2 in fruits of control plants at ripening stages as compared with that during fruit growth (Table 2). Also the decrease in H_2O_2 in fruits stressed at ripening stages may be related to the increase in the antioxidant enzymes in these fruits, suggested that water stress-induced H_2O_2 formation is associated with a parallel increases in activities of CAT and APX occur and contribute to lower H_2O_2 content. Iturbe-Ormaetxe et al. (1998) suggested that there is a relationship between the level of antioxidant enzymes and the rate of ROS production during water stress.

Fig. 5 (continued)

The oxidation of membrane lipids is a reliable indication of uncontrolled free-radical production and hence of oxidative stress (Noctor and Foyer 1998). MDA content of fruits was estimated by the TBARS test. In general, the results obtained show that the increase in MDA content depended on fruit age and the intensity and the duration of water stress (Tables 2 and 3). These results agree with previous results obtained in fruits of tomato cultivar Raissa, where water stress caused a significant increase in H_2O_2 and MDA contents of fruits (Murshed et al. 2008a). Under abiotic stress environments, the plant lipid metabolism is interrupted as a result of oxidative

damage to membrane lipids by ROS and lipid peroxidation (Elkahoui et al. 2005).

Efficient destruction of ROS in plant cells requires the concerted action of antioxidants. Among the non-enzymatic antioxidants, AsA is found to be one of the best characterized compounds, required for many key metabolic functions in plant cells (Foyer 1993). In addition to its ability to directly scavenge ROS, ascorbate together with APX, MDHAR and DHAR participates in one of the major H_2O_2 -scavenging pathways in plant cells the ascorbate-glutathione cycle. In plant cells, the most important reducing substrate for H_2O_2 scavenging is AsA, a high level of AsA is essential effectively to maintain the antioxidant system that protects plants from oxidative damage due to the biotic and abiotic stresses (Reddy et al. 2004). The beneficial effects of AsA in mitigating partially or completely the adverse effects of drought stress may be one aspects of the role of these in the activation of some enzymatic reactions (Kefeli 1981). It is generally assumed that plant tolerance to environmental stresses is positively correlated with AsA content (Knörzer et al. 1996; Tambussi et al. 2000). In our study, the degree to which the AsA and DHA concentrations changed under water stress is extremely variable among different water stress treatments and different fruit development stages (Figs. 1 and 2). These results agree with our previous results, where water stress caused changes in AsA and DHA concentrations in tomato fruits (Murshed et al. 2008a). These changes in AsA and DHA concentrations in fruits under water stress treatments would occur because of either changes in AsA synthesis or changes in AsA regeneration from DHA, which can result from water stress-induced changes in MDHAR and DHAR activities (Jiménez et al. 2002). Given that ascorbate can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via the APX reaction (Noctor and Foyer 1998), the increase of AsA concentration in tomato plants suggests an important role for this antioxidant against oxidative stress provoked by water stress.

It is vital for plants to adjust the enzymatic and non-enzymatic antioxidant systems to control the amounts of ROS to avoid oxidative stress (Allen 1995). The regulation of the activities of SOD, CAT and ascorbate-glutathione cycle enzymes is a rapid and efficient response to limit the excess of ROS generated by environmental stresses and it was observed in different fruits (Scebba et al. 2001; Sofu et al. 2004). An excess of reducing power, with the consequent increase in H_2O_2 and other ROS concentration, likely caused changes in the regulation of some antioxidant enzymes during the water stress period. The antioxidant enzymes play different and complementary roles in the concerted cell defence, such as direct scavenging of ROS (Palatnik et al. 1999). In plants, this enzymatic response could constitute an adaptive advantage in the protection from oxidative

The $O_2^{\cdot-}$ radical is the main source of oxidative injury in plants and several works have been carried out to establish the role of its terminator systems in relation to water stress tolerance (Scandalios 1997). The dismutation of $O_2^{\cdot-}$ into H_2O_2 reaction catalyzed by the SOD maintains intracellular $O_2^{\cdot-}$ within normal levels and various authors have implicated this enzyme in the protection of cells from oxidative stress (Smirnoff 1993). In fact, our results show that SOD activity was increased by some water stress treatments depending on the development stage but the increase was clearly apparent at the breaker stage (Fig. 3a). In this respect, the activation of SOD in stressed plants may be interpreted as a direct response to augmented $O_2^{\cdot-}$ generation. SOD induction by different environmental stresses has been reported (Bowler et al. 1992). It was also reported that the increase in SOD activity enhance plants water stress tolerance (Manivannan et al. 2007c). In tomato plants, SOD activity was induced strongly by water stress (Noctor et al. 2000).

It is therefore essential for plants to have an effective means to detoxify H_2O_2 , a task that can be accomplished by CAT (among other enzymes). CAT catalyses the dismutation of H_2O_2 (Fornazier et al. 2002), and the combined action of CAT and SOD converts the toxic $O_2^{\cdot-}$ into H_2O_2 then into water and molecular oxygen, averting the cellular damage under unfavourable conditions like water stress (Reddy et al. 2004). Our results show, as in case of SOD, a marked increase in CAT activity in fruits at the breaker stage under water stress. Whereas, in the fruits at other development stages, changes in CAT activity were depending on the intensity and duration of water stress (Fig. 3b). Increased CAT activity has been reported under different stress conditions in different plants (Shaoyun 1997; Kerdnaimongkol et al. 1997; Jaleel et al. 2007).

It is apparent that APX has numerous isoenzymes in various plant cell compartments. APX isozymes, able to scavenge the H_2O_2 produced by SOD, are generally located in chloroplasts, but microsomal, peroxisomal and membrane bound forms, as well as soluble cytosolic and apoplastic isozymes, also exist (Noctor and Foyer 1998). Because determination of enzymatic activity of the several isoforms is not easy, the relative transcript levels of some genes encoding these enzymes: cytosolic APX (*SLAPX_{cyto}*), thylakoid-bound APX (*SLAPX_t*), *SIDHAR1*, *SIMDHAR*, cytosolic GR (*SIGR_{cyto}*) and chloroplastic GR (*SIGR_{cp}*) were also analysed under water and salt stresses (Fig. 5). In our study, changes in APX activity and transcript levels in fruits vary according to the development stage and the intensity and duration of water stress. The increase in APX activity was specially noted in fruits at mature green and breaker stages (Fig. 4a). *SLAPX_{cyto}* and *SLAPX_t* transcripts increased under most water stress treatments in fruits at all development stages (Fig. 5a and b). This increase in *SLAPX_{cyto}* and *SLAPX_t* transcripts was not correlated with changes in APX activity, suggested a post-

transcription regulation. Ishikawa and Shigeoka (2008) reported that the expression of chloroplastic APX is post-transcriptionally regulated by alternative splicing. Numerous studies of ROS-scavenging enzymes in plants have demonstrated that APX activity generally increases along with activities of other enzymes, such as CAT and SOD, in response to environmental stress (Shigeoka et al. 2002). Among APX isoenzymes, cytosolic APX is known to be highly responsive to environmental conditions. The steady-state level of the *APX_{cyto}* transcript in pea and spinach leaves increased in response to high-light illumination, water stress, heat, and methyl viologen treatment (Mittler and Zilinskas 1992; Yoshimura et al. 2000). A recent transcriptome-based analysis also confirms the susceptibility of *APX_{cyto}* to environmental factors (Kim et al. 2007). *APX_t* transcript level change also in response to environmental stress (Tanabe et al. 2007).

The regeneration of ascorbate is a process of great importance in the antioxidant response to stress. APX utilizes AsA as its electron donor to reduce H_2O_2 to water with the concomitant generation of monodehydroascorbate (MDHA), a univalent oxidant of AsA. MDHA is either disproportionated to AsA and DHA or reduced to AsA by MDHAR. DHA is also reduced back to AsA by the action of GSH-dependent DHAR, and the resulting oxidized GSH is then regenerated by GR (Asada 1999). Our results show that DHAR activity was increased in fruits by some water stress treatments especially in fruits at ripening stages (Fig. 4b). They also show that *SIDHAR1* and *SIDHAR2* transcripts were increased in fruits by most water stress treatments (Fig. 5c and d). However, changes in DHAR activity in response to water stress were not directly related to changes in *SIDHAR1* and *SIDHAR2* genes expression, suggested a post-transcription regulation or the existence of other genes coding for DHAR.

MDHAR, a FAD enzyme that catalyses the reduction of the MDHA radical, is one of the key enzymes in the ascorbate–glutathione cycle. In plants, MDHAR activity has been detected in the chloroplast, mitochondria, peroxisome, cytosol, and associated with the plasma membrane and cell wall (Dalton et al. 1993; Leonardi et al. 1995; Bérczi and Møller 1998; Mittova et al. 2000). In this study, changes in MDHAR activity were varies depending on the development stage and the intensity and duration of water stress, with a marked increase in fruits at the mature green stage of plants stressed for 6 and 10 days (Fig. 4c). This can suggest that the AsA regeneration by MDHAR in fruits at this stage becomes more important under water stress. *SIMDHAR* transcript increased in fruits of plants stressed at all development stages, except in fruits at 25 DAF where it decreased (Fig. 5e). Jiménez et al. (2002) was reported that *MDHAR* transcript correlated well with MDHAR activity; whereas, in our study we did not find a correlation between MDHAR activity and *SIMDHAR* transcript accumulation under water stress, suggested a post-transcription regulation.

GR regenerate reduced glutathione (GSH), which is utilized as reductant by DHAR. It is present in several isoforms located in different cell compartments. No changes in GR activity was found with water stress treatments in fruits at 25 and 30 DAF. However, in fruits at other development stages GR activity increased under some water stress treatments, especially in fruits at 35 DAF and breaker stage where the increase reached a maximum (Fig. 4d). *SIGRcyto* and *SIGRcp* transcripts were increased by most water stress treatments (Fig. 5f and g). The changes in GR activity and *SIGRcyto* and *SIGRcp* transcripts under water stress were not correlated, suggested a post-transcription regulation (Fidalgo et al. 2011).

From these results, it can be concluded that a relationship can exist between water stress and oxidative stress occurring in tomato fruits, as has also been postulated for other plant species (Iturbe-Ormaetxe et al. 1998; Borsani et al. 2001; Rahman et al. 2002). We have found evidence for changes in ROS scavenging enzymes and ascorbate pool in plants under water stress conditions. We can also confirm that different stress levels at different development stages play a key role in the regulation of ascorbate–glutathione cycle, causing different antioxidant responses. The results obtained in this investigation underline the important role of some antioxidant enzymes and compounds in protecting cellular apparatus during water stress conditions and may be important for the selection for drought resistance in tomato.

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